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A new species of the horned toad *Megophrys* Kuhl & Van Hasselt, 1822 (Anura, Megophryidae) from southwest China

Ning Xu[1*], Shi-Ze Li[1,2,3*], Jing Liu[3], Gang Wei[1], Bin Wang[1,2]

1 *Biodiversity Conservation Key Laboratory, Guiyang College, Guiyang, 550002, China* **2** *CAS Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China* **3** *Department of Food Science and Engineering, Moutai Institute, Renhuai 564500, China*

Corresponding author: Bin Wang (wangbin@cib.ac.cn)

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Abstract

A new species of the genus *Megophrys* is described from Guizhou Province, China. Molecular phylogenetic analyses based on mitochondrial DNA and nuclear DNA sequences all strongly supported the new species as an independent clade sister to *M. minor* and *M. jiangi*. The new species could be distinguished from its congeners by a combination of the following characters: body size moderate (SVL 43.4–44.1 mm in males, and 44.8–49.8 mm in females; vomerine teeth absent; tongue not notched behind; a small horn-like tubercle at the edge of each upper eyelid; tympanum distinctly visible, rounded; two metacarpal tubercles on palm; relative finger lengths II < I < V < III; toes without webbing; heels overlapping when thighs are positioned at right angles to the body; tibiotarsal articulation reaching the level between tympanum and eye when leg stretched forward; in breeding males, an internal single subgular vocal sac in male, and the nuptial pads with black spines on dorsal surface of bases of the first two fingers.

Keywords

Taxonomy, molecular phylogenetic analysis, morphology

* Contributed equally as the first authors.

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Introduction

The Asian horned toad *Megophrys* Kuhl & Van Hasselt, 1822 (Anura: Megophryidae Bonaparte, 1850) is widely distributed in eastern and central China, throughout southeastern Asia, and extending to the islands of the Sunda Shelf and the Philippines (Frost 2020). The taxonomic arrangements especially on generic assignments of the group have been controversial for a long time (e.g., Tian and Hu 1983; Dubois 1987; Lathrop 1997; Rao and Yang 1997; Jiang et al. 2003; Delorme et al. 2006; Fei et al. 2009; Chen et al. 2016; Fei and Ye 2016; Deuti et al. 2017; Mahony et al. 2017; Frost 2019). Nevertheless, all molecular phylogenetic studies revealed this group as a monophyletic group which corresponds to the family (Chen et al. 2016; Mahony et al. 2017; Liu et al. 2018; Li et al. 2018; Liu et al. 2020; Wang et al. 2020), and thus many researchers considered it as a large genus *Megophrys sensu lato* (Mahony et al. 2017; Li et al. 2018; Liu et al. 2018, 2020; Frost 2020; Wang et al. 2020) although several studies divided the taxa of the group into different genera and subgenera, thus introducing better resolution of relationships within the family (Chen et al. 2016; Fei and Ye 2016; Deuti et al. 2017; Liu et al. 2018).

The large genus *Megophrys* currently contains 98 species, of which 41 species were described in the last decade (Frost 2020; Liu et al. 2020). Many cryptic species in the genus are indicated by molecular phylogenetic analyses (Chen et al. 2016; Liu et al. 2018) of which several have been described recently (e.g., Wang et al. 2019; Liu et al. 2020). Obviously, more cryptic species need to be verified and described in detail.

During field surveys in the Chishui National Nature Reserve, Chishui City, Guizhou Province, China, we collected a series of *Megophrys* specimens. Our molecular phylogenetic analyses and morphological comparisons support it as an undescribed species, and it is described herein as a new species.

Materials and methods

Sampling

Three adult males and five adult females of the undescribed species were collected in Chishui National Nature Reserve, Chishui City, Guizhou Province, China (Suppl. material 1: Table S1; Fig. 1). In the field, the toads were euthanized using isoflurane, and the specimens were fixed in 75% ethanol. Tissue samples were taken and preserved separately in 99% ethanol prior to fixation. The specimens were deposited in Chengdu Institute of Biology, Chinese Academy of Sciences (**CIB, CAS**).

Molecular data and phylogenetic analyses

Six specimens of the undescribed species were included in the molecular analyses (Suppl. material 2: Table S2). Total DNA was extracted using a standard phenol-chloroform extraction protocol (Sambrook et al. 1989). Two fragments of the mitochondrial genes encoding16S rRNA and cytochrome oxidase subunit I (COI) were amplified

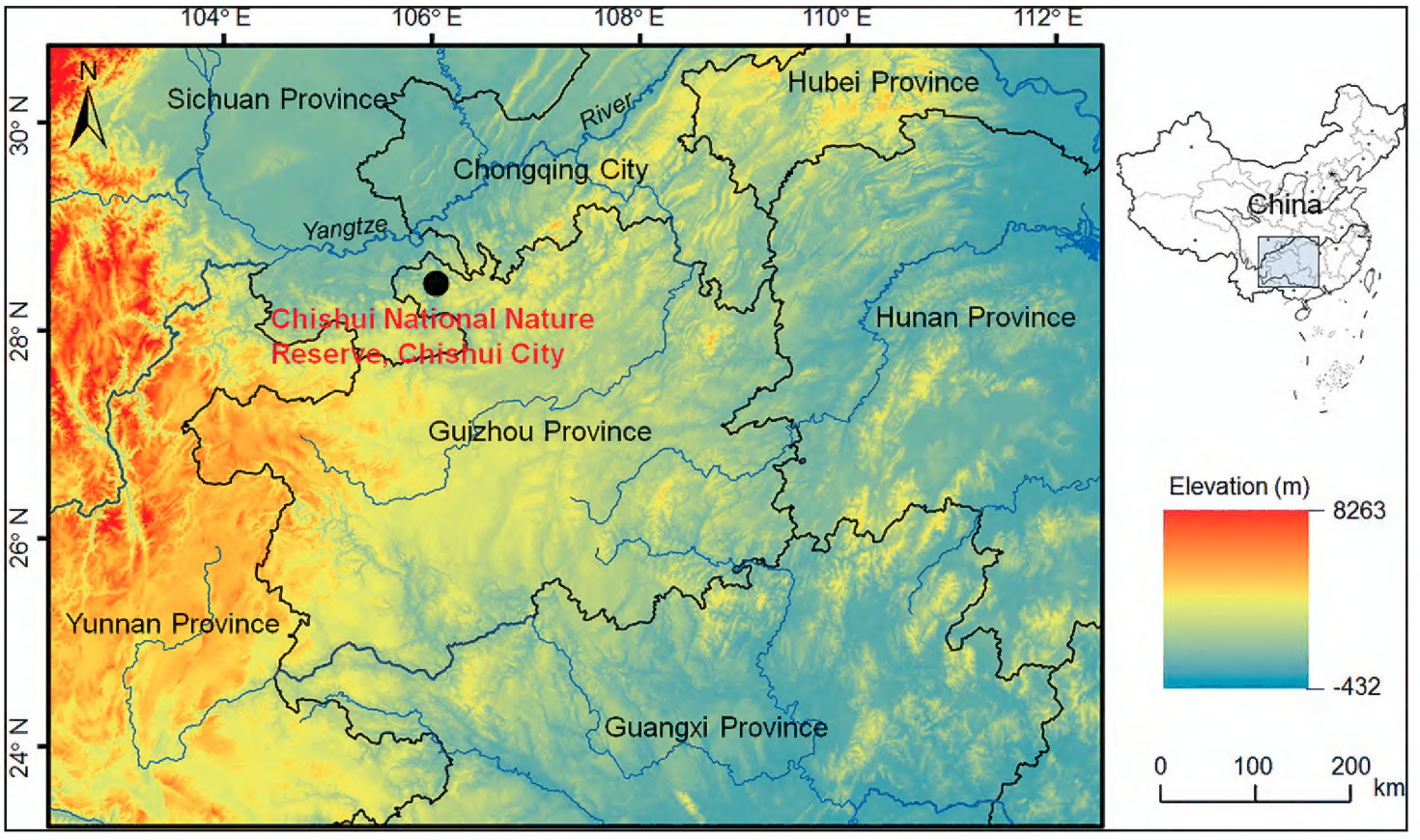

Figure 1. Geographical location of the type locality, Chishui National Nature Reserve, Chishui City, Guizhou Province, China, of *Megophrys chishuiensis* sp. nov.

using the primers in Simon et al. (1994) and Che et al. (2012), respectively. PCR were under the following conditions: 37 cycles at 94 °C for 4 min, 95 °C for 1 min, 53 °C (for 16S rRNA)/47 °C (for COI) for 30 sec, and 72 °C for 1 min followed by a 8-min extension at 72 °C. The nuclear gene sequences encoding brain-derived neurotrophic factor (BDNF) and recombination activating gene 1 (RAG1) were amplified using the primers and protocols in Vieites et al. (2007) and Shen et al. (2013), respectively (Suppl. material 3: Table S3). All PCR products were purified with spin columns, and then were sequenced with primers same as used in PCR. Sequencing was conducted using an ABI3730 automated DNA sequencer in Shanghai DNA BioTechnologies Co., Ltd. (Shanghai, China). All sequences were deposited in GenBank (for accession numbers see Suppl. material 2: Table S2).

For molecular analyses, the available sequence data for congeners of *Megophrys* were downloaded from GenBank (Suppl. material 2: Table S2), primarily from previous studies (Chen et al. 2017; Liu et al. 2018). For phylogenetic analyses, corresponding sequences of one *Leptobrachella oshanensis* (Liu, 1950) and one *Leptobrachium boringii* (Liu, 1945) were also downloaded (Suppl. material 2: Table S2), and used as outgroups according to Mahony et al. (2017). Sequences were assembled and aligned in BioEdit v. 7.0.9.0 (Hall 1999) with default settings. Alignments were checked by eye and revised manually if necessary. To avoid bias in alignments, GBLOCKS v. 0.91.b (Castresana 2000) with default settings was used to extract regions of defined sequence conservation from the length-variable 16S gene fragments. Non-sequenced fragments were defined as missing loci. For phylogenetic analyses, two datasets were obtained, i.e., two-mitochondrial genes concatenated dataset of 16S+COI and two-nuclear genes concatenated dataset of RAG1+BDNF.

Phylogenetic relationships were reconstructed based on the mitochondrial DNA data and nuclear DNA data, respectively. Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian Inference (BI) methods, implemented in PhyML v. 3.0 (Guindon et al. 2010) and MrBayes v. 3.12 (Ronquist and Huelsenbeck 2003), respectively. To avoid under- or over-parameterization (Lemmon and Moriarty 2004; McGuire et al. 2007), the best partition scheme and the best evolutionary model for each partition were chosen for the phylogenetic analyses using PARTITIONFINDER v. 1.1.1 (Robert et al. 2012). In the analyses, 16S, each codon position of the protein-coding genes (COI, RAG1 and BDNF) were defined, and Bayesian Inference Criteria (BIC) was used. As a result, the analyses selected the best partition scheme (i.e., 16S gene/each codon position of COI gene) and the GTR+ G + I model for each partition for mitochondrial DNA dataset, and as well, selected the best partition scheme (i.e., each codon position of RAG1 and BDNF genes) and the GTR+ G + I as the best model for all codon position of RAG1 and BDNF genes. For the ML tree, branch supports were drawn from 10000 non-parametric bootstrap replicates. In BI analyses, two runs each with four Markov chains were run for 40 million generations with sampling every 1000 generations. The first 25% of generations were removed as the "burn-in" stage followed by calculation of Bayesian posterior probabilities and the 50% majority-rule consensus of the post burn-in trees sampled at stationarity. Finally, genetic distance between species under uncorrected *p*-distance model was estimated on 16S gene sequences using MEGA v. 6.06 (Tamura et al. 2011).

Morphological comparisons

All adult specimens of the undescribed species were measured. The terminology and methods followed Fei et al. (2009). Measurements were taken with a dial caliper to 0.1 mm. Seventeen morphometric characters of adult specimens were measured:

ED eye diameter (distance from the anterior corner to the posterior corner of the eye);
FL foot length (distance from tarsus to the tip of fourth toe);
HDL head length (distance from the tip of the snout to the articulation of jaw);
HDW maximum head width (greatest width between the left and right articulations of jaw);
HLL hindlimb length (maximum length from the vent to the distal tip of the Toe IV);
IND internasal distance (minimum distance between the inner margins of the external nares);
IOD interorbital distance (minimum distance between the inner edges of the upper eyelids);
LAL length of lower arm and hand (distance from the elbow to the distal end of the Finger IV);
LW lower arm width (maximum width of the lower arm);
SL snout length (distance from the tip of the snout to the anterior corner of the eye);

SVL snout-vent length (distance from the tip of the snout to the posterior edge of the vent);
TFL length of foot and tarsus (distance from the tibiotarsal articulation to the distal end of the Toe IV);
THL thigh length (distance from vent to knee);
TL tibia length (distance from knee to tarsus);
TYD maximal tympanum diameter;
TW maximal tibia width;
UEW upper eyelid width (greatest width of the upper eyelid margins measured perpendicular to the anterior-posterior axis).

We compared morphological characters of the undescribed species with *Megophrys* congeners. Comparative data were obtained from related species as described in literature (Table 2).

Bioacoustics notes

Ten advertisement calls from two individuals of the new species were recorded on 18 May 2018 between 21:00–23:00 in Chishui City, Guizhou Province, China in the field. SONY PCM-D50 digital sound recorder was used to record within 20 cm of the calling individuals. The sound files in wave format were resampled at 48 kHz with sampling depth 24 bits. The sonograms and waveforms were generated by WaveSurfer software (Sjöander and Beskow 2000) from which all parameters and characters were measured. Ambient temperature was taken by a digital hygrothermograph.

Table 1. Measurements of the adult specimens of *Megophrys chishuiensis* sp. nov. Units are given in mm. See abbreviations for the morphological characters in Materials and methods section.

	Male (N = 3)		Female (N = 5)	
	Range	**Mean ± SD**	**Range**	**Mean ± SD**
SVL	43.4–44.1	43.6 ± 0.4	44.8–49.8	47.8 ± 2.0
HDL	11.4–11.9	11. 7 ± 0.3	11.2–12.7	11.7 ± 0.6
HDW	13.0–13.9	13.5 ± 0.5	13.8–15.4	14.7 ± 0.6
SL	4.2–5.3	4.8 ± 0.6	4.3–5.4	4.9 ± 0.4
IND	5.0–5.2	5.1 ± 0.1	4.5–5.8	5.1 ± 0.6
IOD	3.1–3.5	3.3 ± 0.2	3.1–4.3	3.5 ± 0.5
ED	4.4–5.0	4.7 ± 0.3	4.9–5.7	5.4 ± 0.3
UEW	4.1–4.9	4.4 ± 0.4	4.1–5.2	4.7 ± 0.4
TYD	2.8–3.5	3.2 ± 0.4	2.2–3.1	2.7 ± 0.3
LAL	18.4–20.0	19.0 ± 0.9	20.3–22.0	21.3 ± 0.7
LW	4.5–4.7	4.6 ± 0.1	3.2–3.6	3.4 ± 0.2
HLL	59.4–65.1	63.0 ± 3.1	64.2–75.6	70.7 ± 4.1
THL	17.2–21.3	19.8 ± 2.2	20.4–23.8	22.1 ± 1.3
TL	18.0–21.7	20.1 ± 1.9	22.0–24.0	23.2 ± 0.8
TW	4.6–5.1	4.9 ± 0.3	5.0–5.8	5.3 ± 0.3
TFL	28.0–30.2	28.9 ± 1.2	30.1–33.0	31.3 ± 1.1
FL	18.5–19.2	18.9 ± 2.3	18.8–22.1	21.0 ± 1.4

Table 2. References for morphological characters for congeners of the genus *Megophrys*.

Species	Literature
M. aceras Boulenger, 1903	Taylor 1962
M. acuta Wang, Li & Jin, 2014	Li et al. 2014
M. ancrae Mahony, Teeling & Biju, 2013	Mahony et al. 2013
M. angka Wu, Suwannapoom, Poyarkov, Chen, Pawangkhanant, Xu, Jin, Murphy & Che, 2019	Wu et al. 2019
M. auralensis Ohler, Swan & Daltry, 2002	Ohler et al. 2002
M. baluensis (Boulenger, 1899)	Boulenger 1899
M. baolongensis Ye, Fei & Xie, 2007	Ye et al. 2007
M. binchuanensis Ye & Fei, 1995	Ye and Fei 1995
M. binlingensis Jiang, Fei & Ye, 2009	Fei et al. 2009
M. boettgeri (Boulenger, 1899)	Fei et al. 2012
M. brachykolos Inger & Romer, 1961	Inger and Romer 1961
M. carinense (Boulenger, 1889)	Fei et al. 2009
M. caobangensis Nguyen, Pham, Nguyen, Luong & Ziegler, 2020	Nguyen et al. 2020
M. caudoprocta Shen, 1994	Fei et al. 2012
M. cheni (Wang & Liu, 2014)	Wang et al. 2014
M. chuannanensis (Fei, Ye & Huang, 2001)	Fei et al. 2012
M. damrei Mahony, 2011	Mahony 2011
M. daweimontis Rao & Yang, 1997	Fei et al. 2012
M. dongguanensis Wang & Wang, 2019	Wang et al. 2019
M. dringi Inger, Stuebing & Tan, 1995	Inger et al. 1995
M. edwardinae Inger, 1989	Inger 1989
M. elfina Poyarkov, Duong, Orlov, Gogoleva, Vassilieva, Nguyen, Nguyen, Nguyen, Che & Mahony, 2017	Poyarkov et al. 2017
M. fansipanensis Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018	Tapley et al. 2018
M. feae Boulenger, 1887	Fei et al. 2009
M. feii Yang, Wang & Wang, 2018	Yang et al. 2018
M. flavipunctata Mahony, Kamei, Teeling & Biju, 2018	Mahony et al. 2018
M. gerti (Ohler, 2003)	Ohler 2003
M. gigantica Liu, Hu & Yang, 1960	Fei et al. 2012
M. glandulosa Fei, Ye & Huang, 1990	Fei et al. 2012
M. hansi (Ohler, 2003)	Ohler 2003
M. himalayana Mahony, Kamei, Teeling & Biju, 2018	Mahony et al. 2018
M. hoanglienensis Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018	Tapley et al. 2018
M. huangshanensis Fei & Ye, 2005	Fei et al. 2012
M. insularis (Wang, Liu, Lyu, Zeng & Wang, 2017)	Wang et al. 2017a
M. intermedia Smith, 1921	Rao and Yang 1997
M. jiangi Liu, Li, Wei, Xu, Cheng, Wang & Wu, 2020	Liu et al. 2020
M. jingdongensis Fei & Ye, 1983	Fei et al. 2012
M. jinggangensis (Wang, 2012)	Wang et al. 2012
M. jiulianensis Wang, Zeng, Lyu & Wang, 2019	Wang et al. 2019
M. kalimantanensis Munir, Hamidy, Matsui, Iskandar, Sidik & Shimada, 2019	Munir et al. 2019
M. kobayashii Malkmus & Matsui, 1997	Malkmus and Matsui 1997
M. koui Mahony, Foley, Biju & Teeling, 2017	Mahony et al. 2017
M. kuatunensis Pope, 1929	Fei et al. 2012
M. lancip Munir, Hamidy, Farajallah & Smith, 2018	Munir et al. 2018
M. leishanensis Li, Xu, Liu, Jiang, Wei & Wang, 2018	Li et al. 2018
M. lekaguli Stuart, Chuaynkern, Chan-ard & Inger, 2006	Stuart et al. 2006
M. liboensis (Zhang, Li, Xiao, Li, Pan, Wang, Zhang & Zhou, 2017)	Zhang et al. 2017
M. ligayae Taylor, 1920	Taylor 1920
M. lini (Wang & Yang, 2014)	Wang et al. 2014
M. lishuiensis (Wang, Liu & Jiang, 2017)	Wang et al. 2017b
M. longipes Boulenger, 1886	Taylor 1962
M. major Boulenger, 1908	Mahony et al. 2018
M. mangshanensis Fei & Ye, 1990	Fei et al. 2012
M. maosonensis Bourret, 1937	Bourret 1937
M. medogensis Fei, Ye & Huang, 1983	Fei et al. 2012
M. megacephala Mahony, Sengupta, Kamei & Biju, 2011	Mahony et al. 2011
M. microstoma (Boulenger, 1903)	Fei et al. 2012
M. minor Stejneger, 1926	Fei et al. 2012
M. montana Kuhl & Van Hasselt, 1822	Kuhl and Van Hasselt 1822

Species	Literature
M. monticola (Günther, 1864)	Mahony et al. 2018
M. mufumontana Wang, Lyu & Wang, 2019	Wang et al. 2019
M. nankiangensis Liu & Hu, 1966	Fei et al. 2012
M. nankunensis Wang, Zeng & Wang, 2019	Wang et al. 2019
M. nanlingensis Lyu, Wang, Liu & Wang, 2019	Wang et al. 2019
M. nasuta (Schlegel, 1858)	Taylor 1962
M. obesa Wang, Li & Zhao, 2014	Wang et al. 2014
M. ombrophila Messenger & Dahn, 2019	Munir et al. 2019
M. omeimontis Liu, 1950	Fei et al. 2009
M. oreocrypta Mahony, Kamei, Teeling & Biju, 2018	Mahony et al. 2018
M. oropedion Mahony, Teeling & Biju, 2013	Mahony et al. 2013
M. orientalis Li, Lyu, Wang & Wang, 2020	Li et al. 2020
M. pachyproctus Huang, 1981	Fei et al. 2009
M. palpebralespinosa Bourret, 1937	Fei et al. 2012
M. parallela Inger & Iskandar, 2005	Inger and Iskandar 2005
M. parva (Boulenger, 1893)	Fei et al. 2009
M. periosa Mahony, Kamei, Teeling & Biju, 2018	Mahony et al. 2018
M. popei (Zhao, Yang, Chen, Chen & Wang, 2014)	Zhao et al. 2014
M. robusta Boulenger, 1908	Mahony et al. 2018
M. rubrimera Tapley, Cutajar, Mahony, Chung, Dau, Nguyen, Luong & Rowley, 2017	Tapley et al. 2017
M. sangzhiensis Jiang, Ye & Fei, 2008	Jiang et al. 2008
M. serchhipii (Mathew & Sen, 2007)	Mathew and Sen 2007
M. shapingensis Liu, 1950	Fei et al. 2009
M. shuichengensis Tian & Sun, 1995	Fei et al. 2009
M. shunhuangensis Wang, Deng, Liu, Wu & Liu, 2019	Wang et al. 2019a
M. spinata Liu & Hu, 1973	Fei et al. 2009
M. stejnegeri Taylor, 1920	Taylor 1920
M. synoria (Stuart, Sok & Neang, 2006)	Stuart et al. 2006
M. takensis Mahony, 2011	Mahony 2011
M. tuberogranulata Shen, Mo & Li, 2010	Fei et al. 2012
M. vegrandis Mahony, Teeling & Biju, 2013	Mahony et al. 2013
M. wawuensis Fei, Jiang & Zheng, 2001	Fei et al. 2012
M. wugongensis Wang, Lyu & Wang, 2019	Wang et al. 2019b
M. wuliangshanensis Ye & Fei, 1995	Fei et al. 2012
M. wushanensis Ye & Fei, 1995	Fei et al. 2012
M. xianjuensis Wang, Wu, Peng, Shi, Lu & Wu, 2020	Wang et al. 2020
M. zhangi Ye & Fei, 1992	Fei et al. 2012
M. zunhebotoensis (Mathew & Sen, 2007)	Mathew and Sen 2007

Results

Phylogenetic analyses

Aligned sequence matrix of 16S+COI and RAG1+BDNF contains 1104 bp and 1582 bp, respectively. ML and BI trees of the mitochondrial DNA dataset presented almost consistent topology (Fig. 2), and as well, ML and BI trees of the nuclear DNA dataset showed almost identical topology (Fig. 3), though relationships of many lineages were unresolved (Figs 2, 3). In mitochondrial DNA trees, the undescribed species was clustered as an independent clade sister to a clade in comprising of *M. minor* Stejneger, 1926 and *M. jiangi* Liu, Li, Wei, Xu, Cheng, Wang & Wu, 2020, but in nuclear DNA trees, the undescribed species clade was sister to *M. jiangi*, and then was clustered together with *M. minor*.

Genetic distances on16S gene with uncorrected *p*-distance model between samples of the undescribed species were below 0.2%. The genetic distance between the undescribed species and its closest related species *M. minor* was 2.2% on 16S gene, which was higher or at the same level with those among many pairs of congeners, for

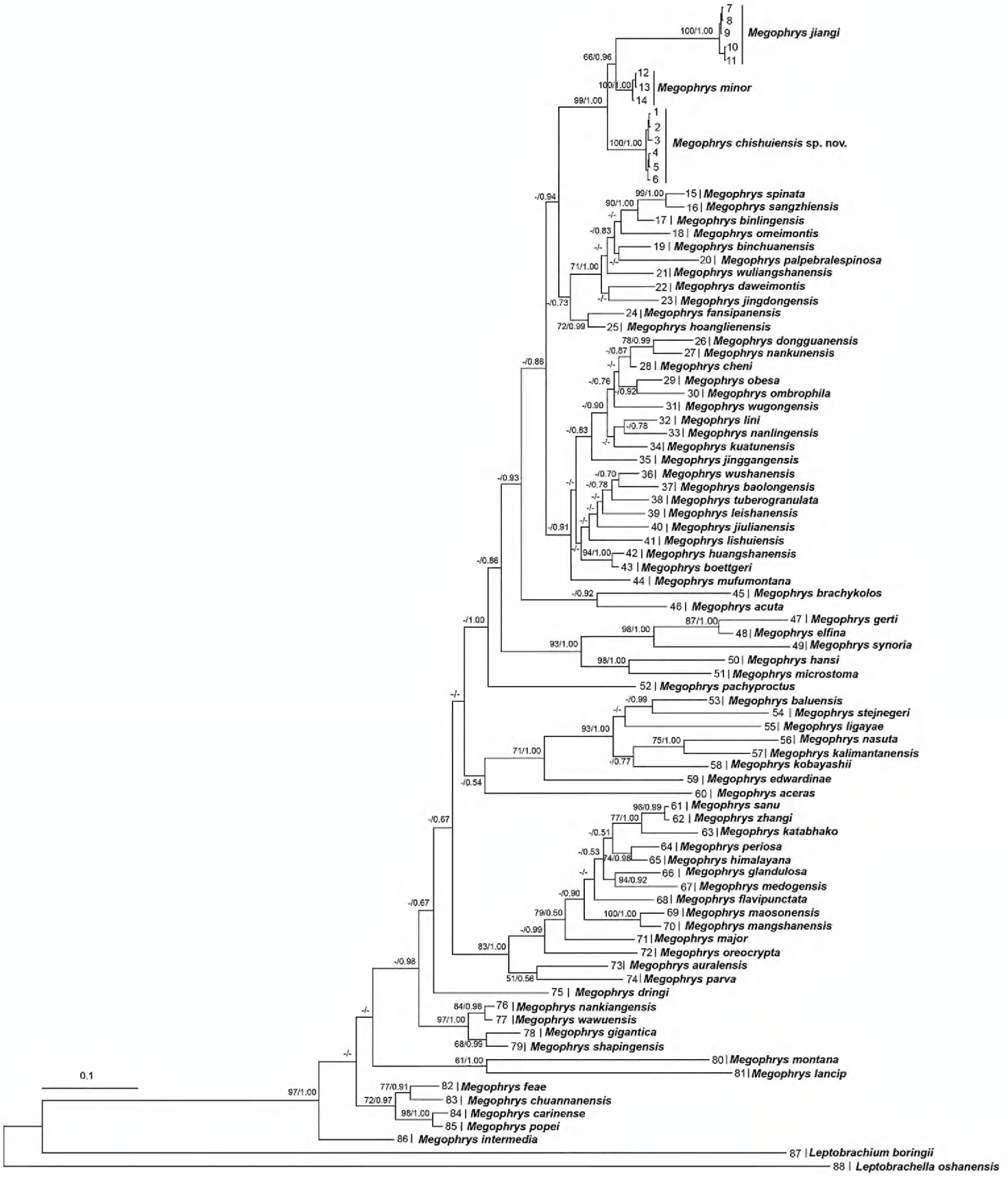

Figure 2. Maximum likelihood (ML) tree of the genus *Megophrys* reconstructed based on the 16S rRNA and COI gene sequences. Bayesian posterior probability/ML bootstrap supports were denoted beside each node. Samples 1–88 refer to Suppl. material 2: Table S2.

example, 1.7% between *M. spinata* Liu & Hu, 1973 and *M. sangzhiensis* Jiang, Ye & Fei, 2008, 2.1% between *M. omeimontis* Liu, 1950 and *M. binlingensis* Jiang, Fei & Ye, 2009, and 2.2% between *M. cheni* (Wang & Liu, 2014) and *M. nankunensis* Wang, Zeng & Wang, 2019; Suppl. material 4: Table S4).

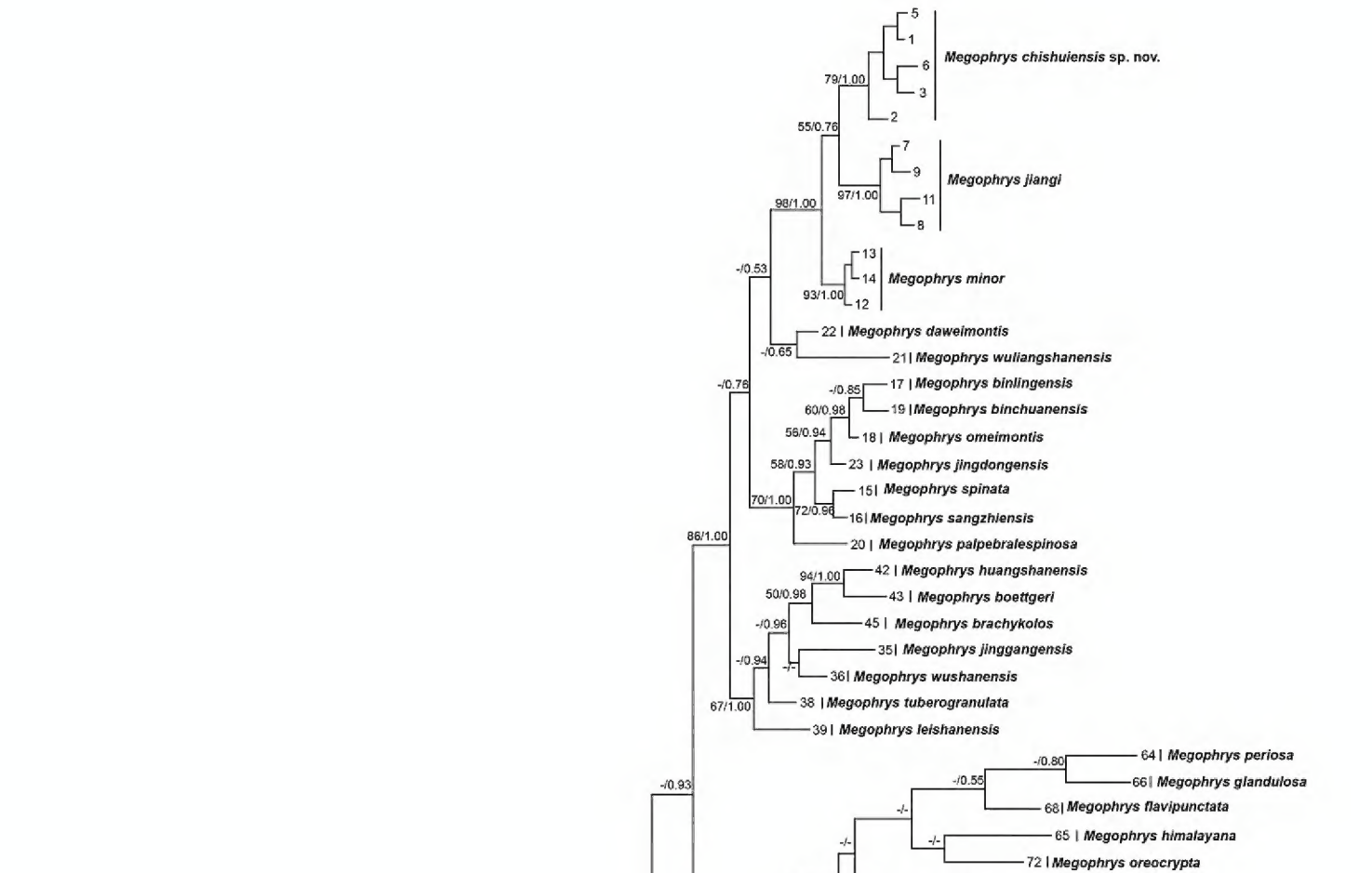

Figure 3. Maximum likelihood (ML) tree of the genus *Megophrys* reconstructed based on the nuclear DNA sequences of RAG1 and BDNF genes. Bayesian posterior probability/ML bootstrap supports were denoted beside each node. Samples 1–88 refer to Suppl. msterial 2: Table S2.

Taxonomic accounts

***Megophrys chishuiensis* sp. nov.**
http://zoobank.org/20B6A80B-E937-4443-88A2-E357B77DB6CA
Figures 4–8

Type material. ***Holotype.*** CIBCS20190518031 (Figs 4, 5), adult male, from Chishui National Nature Reserve, Chishui City, Guizhou Province, China (28.436708N, 105.997794E, ca. 460 m a. s. l.), collected by Shi-Ze Li on 18 May 2019.

Paratype. Two adult males and five adult females from the same place as holotype, collected by Shi-Ze Li and Jing Liu. Two females CIBCS20190518022 and CIBCS20190518023 collected by Jing LIU on 18 May 2019, two adult males CIBCS20190518019 and CIBCS20190518021 and three adult females CIBCS20190518025, CIBCS20190518027 and CIBCS20190518030 collected by Shi-Ze Li on 18 May 2019.

Diagnosis. *Megophrys chishuiensis* sp. nov. is assigned to the genus *Megophrys* based on molecular phylogenetic analyses and the following generic diagnostic characters: snout shield-like; projecting beyond the lower jaw; canthus rostralis distinct; chest glands small and round, closer to the axilla than to midventral line; femoral glands on rear part of thigh; vertical pupils (Fei et al. 2009).

Megophrys chishuiensis sp. nov. could be distinguished from its congeners by a combination of the following morphological characters: (1) body size moderate (SVL 43.4–44.1 mm in males, and 44.8–49.8 mm in females; (2) vomerine teeth absent; (3) tongue not notched behind; (4) a small horn-like tubercle at the edge of each upper eyelid; (5) tympanum distinctly visible, rounded; (6) two metacarpal tubercles on palm; (7) relative finger lengths II < I < V < III; (8) toes without webbing; (9) heels overlapping when thighs are positioned at right angles to the body; (10) tibiotarsal articulation reaching the level between tympanum and eye when leg stretched forward. In breeding male, (11) an internal single subgular vocal sac; (12) nuptial pads with black spines on dorsal surface of bases of the first two fingers.

Description of holotype. (Figs 4, 5). SVL 43.4 mm; head width larger than head length (HDW/HDL ratio about 1.2); snout obtusely pointed, protruding well beyond the margin of the lower jaw in ventral view; loreal region vertical and concave; canthus rostralis well-developed; top of head flat in dorsal view; a small horn-like tubercle at the edge of the upper eyelid; eye large, eye diameter 43.9% of head length; pupils vertical; nostril orientated laterally, closer to snout than eye; tympanum distinct, TYP/EYE ratio 0.64; vomerine ridges and vomerine teeth absent; margin of tongue smooth, not notched behind.

Forelimbs slender, the length of lower arm and hand 42.4% of SVL; fingers slender, relative finger lengths: II < I < V < III; tips of digits globular, without lateral fringes; subarticular tubercle distinct at the base of each finger; two metacarpal tubercles, prominent, the outer one long and thin, the inner one oval-shaped.

Hindlimbs slender, 1.48 times SVL; heels overlapping when thighs are positioned at right angles to the body, tibiotarsal articulation reaching tympanum to eye when leg stretched forward; tibia length longer than thigh length; relative toe lengths I < II < V < III < IV; tips of toes round, slightly dilated; subarticular tubercles absent; toes without webbing; no lateral fringe; inner metatarsal tubercle oval-shaped; outer metatarsal tubercle absent.

Dorsal skin rough, with numerous granules; several large warts scattered on flanks; a small horn-like tubercle at the edge of each upper eyelid; tubercles on the dorsum forming a weak X-shaped ridge, the V-shaped ridges disconnect; two discontinuous dorsolateral parallel ridges on either side of the X-shaped ridges; an inverted triangular brown speckle between two upper eyelids; several tubercles on the flanks and dorsal

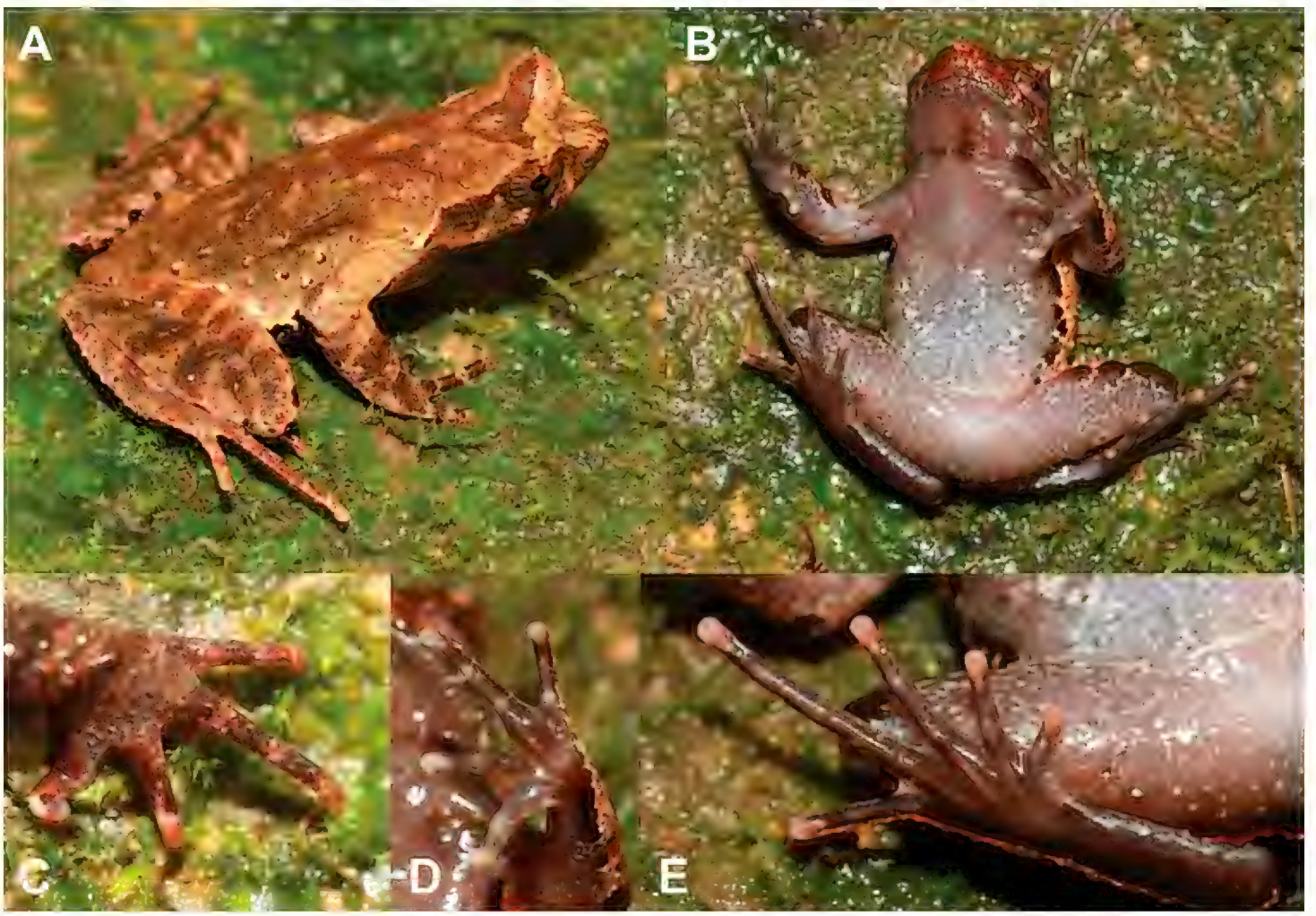

Figure 4. Photos of the holotype CIBCS20190518031 of *Megophrys chishuiensis* sp. nov. in life **A** dorsal view **B** ventral view **C** dorsal view of hand **D** ventral view of hand **E** ventral view of foot.

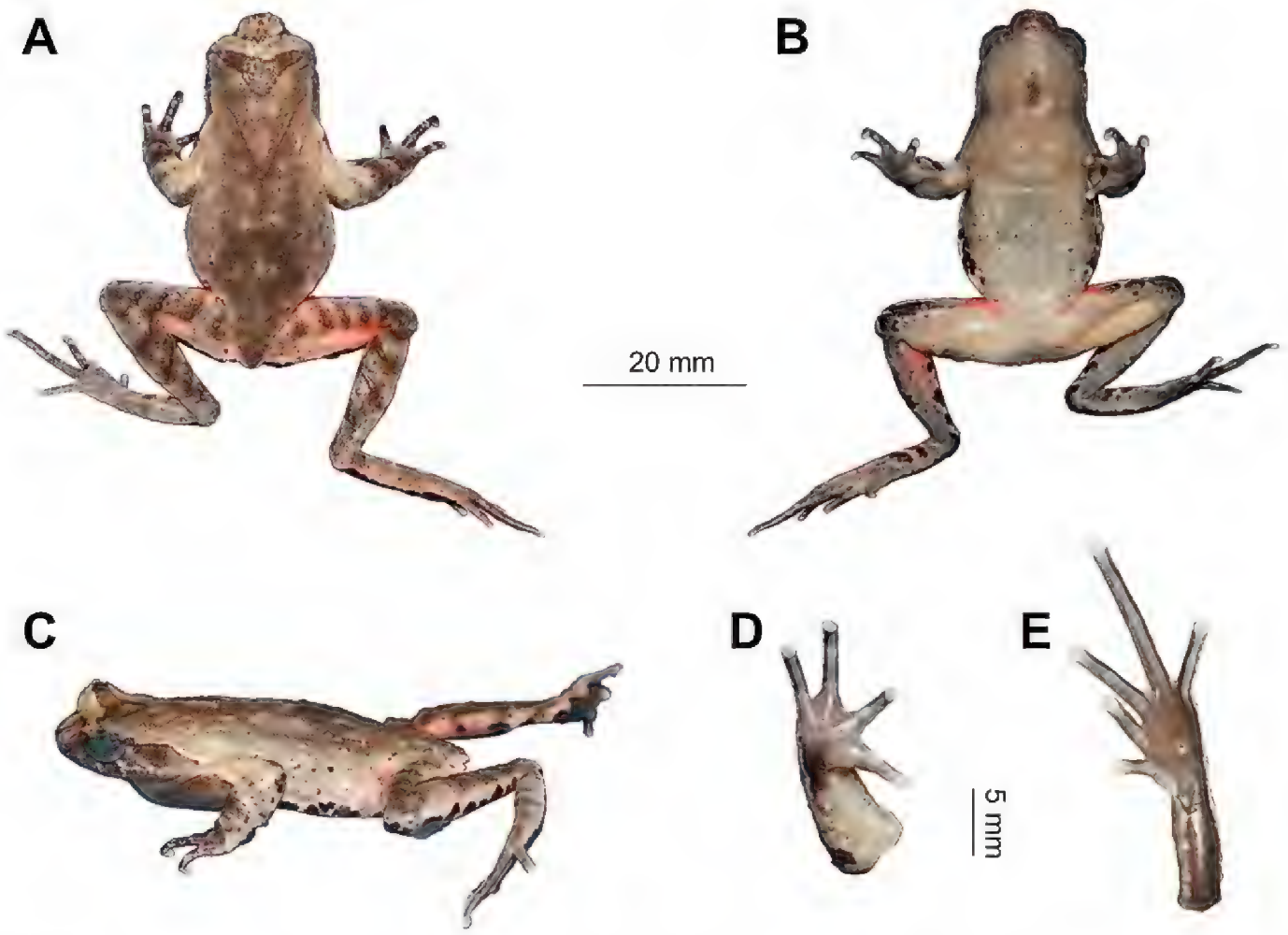

Figure 5. The holotype specimen CIBCS20190518031 of *Megophrys chishuiensis* sp. nov. **A** dorsal view **B** ventral view **C** lateral view **D** ventral view of hand **E** ventral view of foot.

surface of thighs and tibias and forming four transverse tubercle rows; supratympanic fold distinct.

Ventral surface smooth; chest with small and round glands, closer to the axilla than to midventral line; femoral glands on rear of thighs, numerous white granules on outer thighs; posterior end of the body distinctly protruding and forming an arc-shaped swelling above the anal region.

Coloration of holotype in life. (Fig. 4). An inverted triangular brown speckle between the eyes; X-shaped ridges on the dorsum, four transverse bands on the dorsal surface of the thigh and shank; several dark brown and white vertical bars on the lower and upper lip; venter purple grey, some white spots on the ventral surface of body and limbs; palms and soles uniform purple grey, tip of digits pinkish; pectoral and femoral glands white.

Coloration of holotype in preservation. (Fig. 5). Color of dorsal surface fades to olive; the inverted triangular brown speckle between the eyes, X-shaped ridges on dorsum and transverse bands on limbs and digits distinct; ventral surface greyish white; creamy-white substitutes the pinkish on tip of digits; the posterior of ventral surface of body, inner of thigh and upper of tibia light red.

Variations. In CIBCS20190518027, the back is brown with some brick-red granules (Fig. 6A); in CIBCS20190518030, the X-shaped marking on back of trunk consists of a ridge with brown spots (Fig. 6B), and the throat and anterior belly are purplish, with grey spots on the posterior belly and black spots on the flank belly (Fig. 6E); in CIBCS20190518025, the marking on the back consists of a V-shaped ridge (Fig. 6C), and the anterior belly is brownish with some black spots on flank and belly, and posterior belly is beige (Fig. 6F); in CIBCS20190518019, the whole ventrum is purplish except the posterior belly that shows white blotches (Fig. 6D).

Advertisement call. The call description is based on recordings of the holotype CIBCS20190518031 (Fig. 7) from the shrub leaf near the streamlet, and the ambient air temperature was 24.5 °C. Each call consists of 14–20 (mean 16.14 ± 1.95, N = 10) notes. Call duration was 2.10–3.18 second (mean 2.51 ± 0.33, N = 7). Call interval was 0.92–1.32 seconds (mean 1.13 ± 0.15, N = 6). Each note had a duration of 0.07– 0.12 seconds (mean 0.98 ± 0.01, N = 113) and the intervals between notes 0.038–0.085 seconds (mean 0.056 ± 0.011, N = 106). Amplitude modulation within note was apparent, beginning with moderately high energy pulses, increasing slightly to a maximum by approximately mid note, and then decreasing towards the end of each note. The average dominant frequency was 5859 ± 118.02.61 (5733–6064 Hz, N = 7).

Secondary sexual characters. Adult females with SVL 44.8–49.8 mm, larger than adult males with 43.4–44.1 mm. Adult males have a single subgular vocal sac. In breeding males, brownish red nuptial pads are present on dorsal surface of the bases of the first and second fingers with black spines obvious under microscope.

Comparisons. By having medium body size, *Megophrys chishuiensis* sp. nov. differs from *M. aceras* Boulenger, 1903, *M. auralensis* Ohler, Swan & Daltry, 2002, *M. carinense* Boulenger, 1889, *M. caudoprocta* Shen, 1994, *M. chuannanensis* (Fei, Ye & Huang, 2001), *M. damrei* Mahony, 2011, *M. edwardinae* Inger, 1989, *M. feae* Boulenger, 1887, *M. flavipunctata* Mahony, Kamei, Teeling & Biju, 2018, *M. gigantica* Liu, Hu

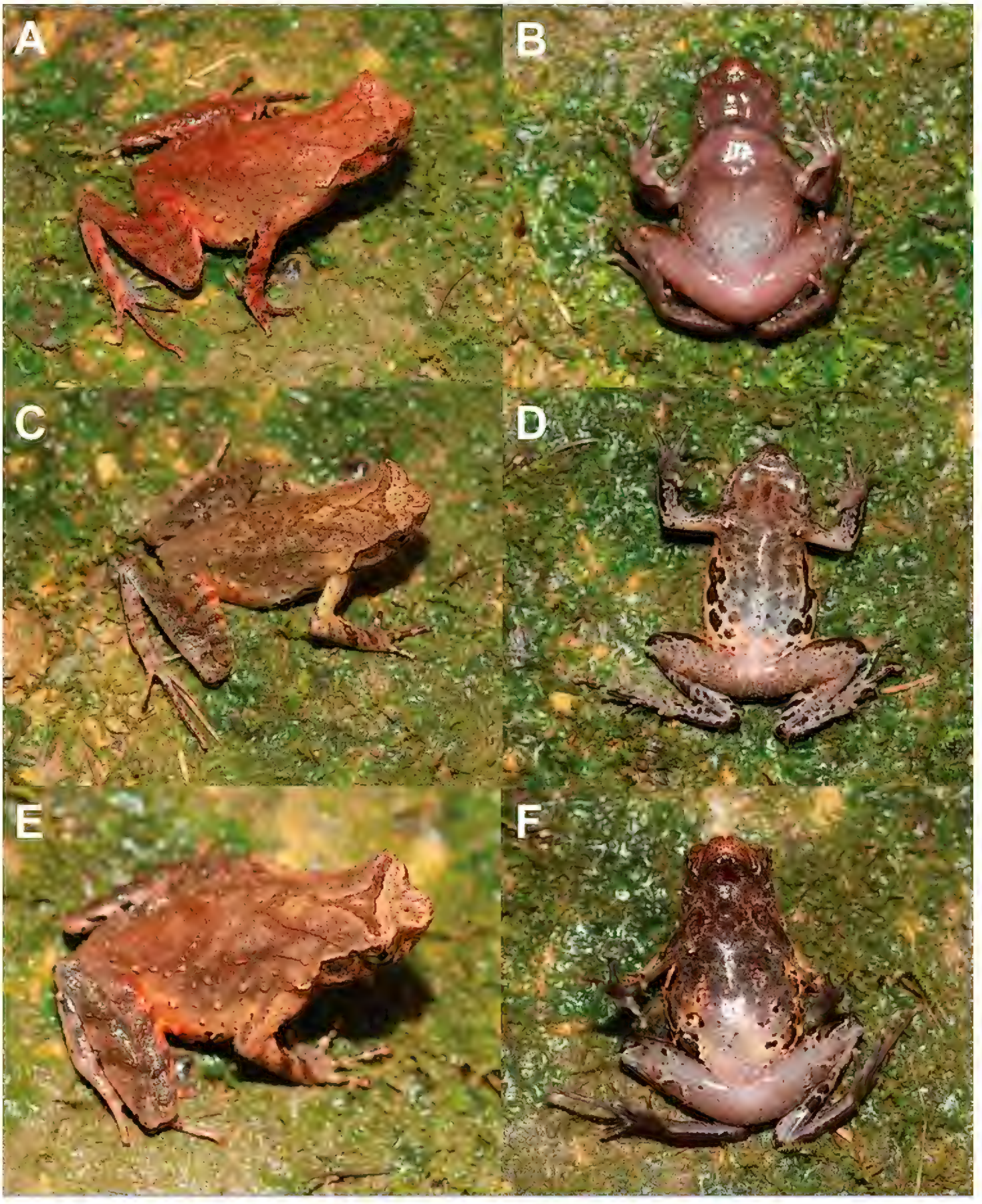

Figure 6. Color variation in *Megophrys chishuiensis* sp. nov. **A** dorsolateral view of the female specimen CIBCS20190518027 **B** dorsolateral view of the female specimen CIBCS20190518030 **C** dorsal view of the female specimen CIBCS20190518025 **D** ventral view of the male specimen CIBCS20190518019 **E** ventral view of the female specimen CIBCS20190518030 **F** ventral view of the female specimen CIBCS20190518025.

& Yang, 1960, *M. glandulosa* Fei, Ye & Huang, 1990, *M. himalayana* Mahony, Kamei, Teeling & Biju, 2018, *M. intermedia* Smith, 1921, *M. jingdongensis* Fei & Ye, 1983, *M. kalimantanensis* Munir, Hamidy, Matsui, Iskandar, Sidik & Shimada, 2019, *M. lekaguli* Stuart, Chuaynkern, Chan-ard & Inger, 2006, *M. liboensis* (Zhang, Li, Xiao,

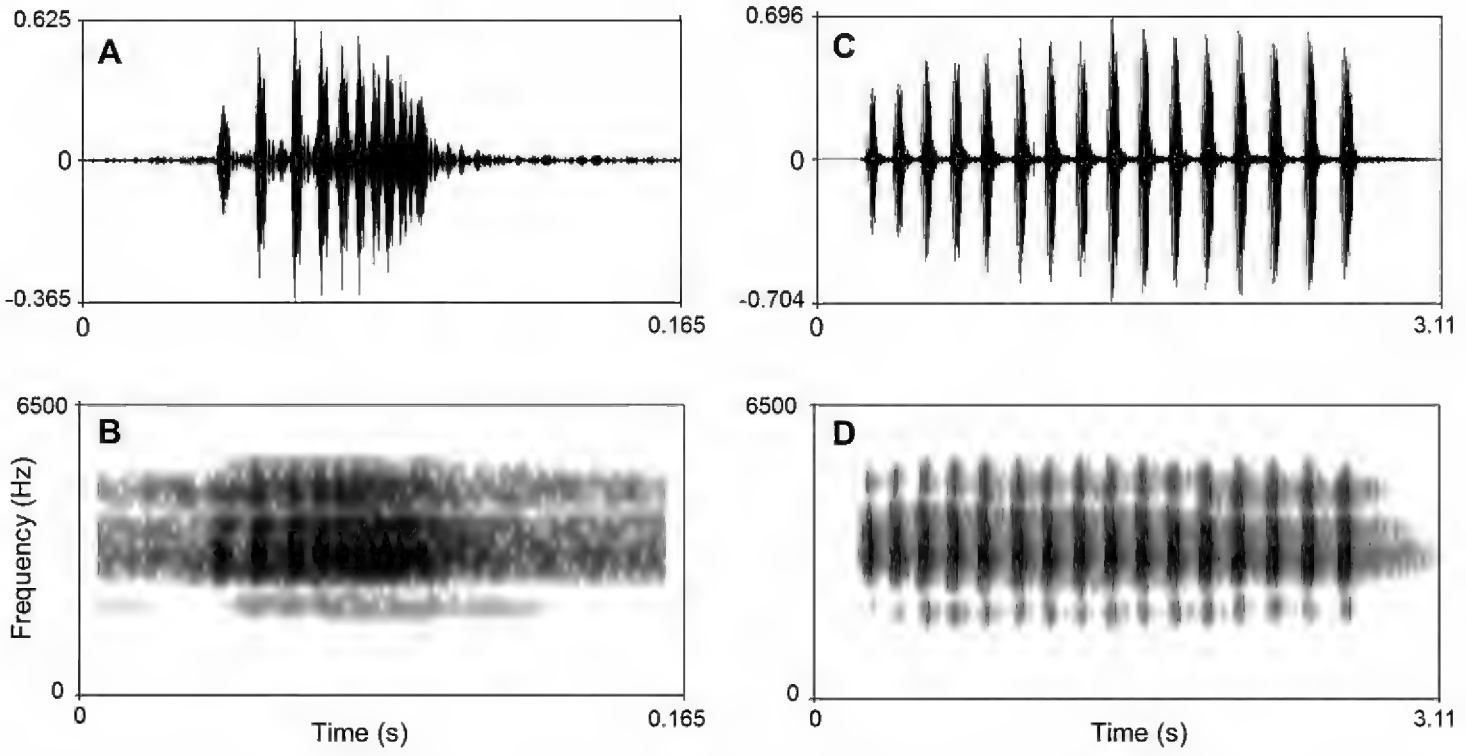

Figure 7. Visualization of advertisement calls of *Megophrys chishuiensis* sp. nov. **A** waveform showing one note **B** sonogram showing one notes **C** waveform showing 16 notes of one call **D** sonogram showing 16 notes of one call.

Li, Pan, Wang, Zhang & Zhou, 2017), *M. major* Boulenger, 1908, *M. mangshanensis* Fei & Ye, 1990, *M. maosonensis* Bourret, 1937, *M. medogensis* Fei, Ye & Huang, 1983, *M. omeimontis* Liu, 1950, *M. oreocrypta* Mahony, Kamei, Teeling & Biju, 2018, *M. orientalis* (Li, Lyu, Wang & Wang, 2020), *M. periosa* Mahony, Kamei, Teeling & Biju, 2018, *M. popei* (Zhao, Yang, Chen, Chen & Wang, 2014), *M. sangzhiensis* Jiang, Ye & Fei, 2008, *M. shapingensis* Liu, 1950, *M. shuichengensis* Tian & Sun, 1995, and *M. takensis* Mahony, 2011 (maximum SVL < 49.8 mm in the new species vs. minimum SVL > 53 mm in the latter), and differs from *M. acuta* Wang, Li & Jin, 2014, *M. angka* (Wu, Suwannapoom, Poyarkov, Chen, Pawangkhanant, Xu, Jin, Murphy & Che, 2019), *M. caobangensis* Nguyen, Pham, Nguyen, Luong & Ziegler, 2020, *M. damrei* Mahony, 2011, *M. dongguanensis* Wang & Wang, 2019, *M. cheni*, *M. jiangi*, *M. jinggangensis* (Wang, 2012), *M. jiulianensis* Wang, Zeng, Lyu & Wang, 2019, *M. kuatunensis* Pope, 1929, *M. lini* (Wang & Yang, 2014), *M. lishuiensis* (Wang, Liu & Jiang, 2017), *M. mufumontana* (Wang, Lyu & Wang, 2019), *M. minor*, *M. nanlingensis* (Lyu, Wang, Liu & Wang, 2019), *M. obesa* Wang, Li & Zhao, 2014, *M. pachyproctus* Huang, 1981, *M. palpebralespinosa* Bourret, 1937, *M. serchhipii* Mathew & Sen, 2007, *M. shunhuangensis* Wang, Deng, Liu, Wu & Liu, 2019, *M. vegrandis* Mahony, Teeling & Biju, 2013, *M. wuliangshanensis* Ye & Fei, 1995, *M. wushanensis* Ye & Fei, 1995, *M. zunhebotoensis* Mathew & Sen, 2007, *M. xianjuensis* Wang, Wu, Peng, Shi, Lu & Wu, 2020, and *M. zhangi* Ye & Fei, 1992 (vs. maximum SVL < 42 mm in the latter).

By the absence of vomerine teeth, *Megophrys chishuiensis* sp. nov. differs from *M. aceras*, *M. ancrae* Mahony, Teeling & Biju, 2013, *M. carinense*, *M. baluensis* (Boulenger, 1899), *M. caudoprocta*, *M. chuannanensis*, *M. damrei*, *M. daweimontis* Rao & Yang, 1997, *M. dongguanensis*, *M. fansipanensis* Tapley, Cutajar, Mahony, Nguyen,

Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018, *M. flavipunctata*, *M. glandulosa*, *M. hoanglienensis* Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018, *M. himalayana*, *M. insularis* (Wang, Liu, Lyu, Zeng & Wang, 2017), *M. intermedia*, *M. jingdongensis*, *M. jinggangensis*, *M. jiulianensis*. *M. kalimantanensis*, *M. kobayashii* Malkmus & Matsui, 1997, *M. lancip* Munir, Hamidy, Farajallah & Smith, 2018, *M. lekaguli*, *M. liboensis*, *M. ligayae* Taylor, 1920, *M. longipes* Boulenger, 1886, *M. major*, *M. mangshanensis*, *M. maosonensis*, *M. medogensis*, *M. megacephala* Mahony, Sengupta, Kamei & Biju, 2011, *M. montana* Kuhl & Van Hasselt, 1822, *M. nasuta* (Schlegel, 1858), *M. nankunensis*, *M. nanlingensis*, *M. omeimontis*, *M. oropedion* Mahony, Teeling & Biju, 2013, *M. oreocrypta*, *M. palpebralespinosa*, *M. parallela* Inger & Iskandar, 2005, *M. parva* (Boulenger, 1893), *M. periosa*, *M. popei*, *M. robusta* Boulenger, 1908, *M. rubrimera* Tapley, Cutajar, Mahony, Chung, Dau, Nguyen, Luong & Rowley, 2017, *M. sangzhiensis*, *M. stejnegeri* Taylor, 1920, *M. takensis*, *M. zhangi*, and *M. zunhebotoensis* (vs. present in the latter).

By having a small horn-like tubercle at the edge of each upper eyelid, *Megophrys chishuiensis* sp. nov. differs from *M. binchuanensis* Ye & Fei, 1995, *M. binlingensis*, *M. damrei*, *M. gigantica*, *M. minor*, *M. monticola* (Günther, 1864), *M. nasuta*, *M. nankiangensis* Liu & Hu, 1966, *M. oropedion*, *M. pachyproctus*, *M. spinata*, *M. stejnegeri*, *M. takensis*, *M. wuliangshanensis*, *M. wushanensis*, *M. zhangi*, and *M. zunhebotoensis* (vs. lacking tubercle in the latter), and differs from *M. carinense*, *M. feae*, *M. gerti* (Ohler, 2003), *M. hansi* (Ohler, 2003), *M. intermedia*, *M. kalimantanensis*, *M. koui* Mahony, Foley, Biju & Teeling, 2017, *M. latidactyla*, *M. liboensis*, *M. microstoma* (Boulenger, 1903), *M. palpebralespinosa*, *M. popei*, *M. shuichengensis*, and *M. synoria* (Stuart, Sok & Neang, 2006) (vs. having a prominent and elongated tubercle in the latter).

By having a tongue not notched behind, *Megophrys chishuiensis* sp. nov. differs from *M. ancrae*, *M. baolongensis* Ye, Fei & Xie, 2007, *M. binlingensis*, *M. boettgeri* (Boulenger, 1899), *M. carinense*, *M. cheni*, *M. chuannanensis*, *M. damrei*, *M. dringi* Inger, Stuebing & Tan, 1995, *M. fansipanensis*, *M. feae*, *M. feii* Yang, Wang & Wang, 2018, *M. flavipunctata*, *M. gerti*, *M. glandulosa*, *M. hoanglienensis*, *M. huangshanensis* Fei & Ye, 2005, *M. insularis*, *M. jiulianensis*. *M. jingdongensis*, *M. kalimantanensis*, *M. kuatunensis*, *M. liboensis*, *M. mangshanensis*, *M. maosonensis*, *M. medogensis*, *M. minor*, *M. nankiangensis*, *M. nanlingensis*, *M. omeimontis*, *M. oropedion*, *M. pachyproctus*, *M. parallela*, *M. popei*, *M. robusta*, *M. sangzhiensis*, *M. shapingensis*, *M. shuichengensis*, *M. spinata*, *M. vegrandis*, *M. wawuensis* Fei, Jiang & Zheng, 2001, *M. zhangi*, and *M. zunhebotoensis* (vs. tongue notched behind in the latter).

By lacking lateral fringes on the toes, *Megophrys chishuiensis* sp. nov. differs from *M. acuta*, *M. auralensis*, *M. baolongensis*, *M. binchuanensis*, *M. boettgeri*, *M. carinense*, *M. cheni*, *M. chuannanensis*, *M. elfina* Poyarkov, Duong, Orlov, Gogoleva, Vassilieva, Nguyen, Nguyen, Nguyen, Che & Mahony, 2017, *M. feae*, *M. feii*, *M. flavipunctata*, *M. gigantica*, *M. glandulosa*, *M. hansi*, *M. intermedia*, *M. jingdongensis*, *M. jinggangensis*, *M. kuatunensis*, *M. latidactyla*, *M. lini*, *M. major*, *M. maosonensis*, *M. nankiangensis*, *M. omeimontis*, *M. palpebralespinosa*, *M. popei*, *M. rubrimera*, *M. sangzhiensis*, *M. serchhipii*,

M. shapingensis, *M. shuichengensis*, *M. spinata*, *M. vegrandis*, *M. xianjuensis*, *M. zhangi*, and *M. zunhebotoensis* (vs. present in these species).

By having toes without webs at bases, *Megophrys chishuiensis* sp. nov. differs from *M. brachykolos* Inger & Romer, 1961, *M. carinense*, *M. flavipunctata*, *M. jingdongensis*, *M. jinggangensis*, *M. lini*, *M. major*, *M. palpebralespinosa*, *M. popei*, *M. shuichengensis*, *M. spinata* (vs. at least one-fourth webbed).

By heels overlapping when thighs are positioned at right angles to the body, *Megophrys chishuiensis* sp. nov. differs from *M. acuta*, *M. brachykolos*, *M. dongguanensis*, *M. huangshanensis*, *M. kuatunensis*, *M. nankunensis*, *M. obesa*, *M. ombrophila* Messenger & Dahn, 2019, and *M. wugongensis* Wang, Lyu & Wang, 2019 (vs. not meeting).

With tibiotarsal articulation reaching to the level between tympanum and eye when leg is stretched forward, *Megophrys chishuiensis* sp. nov. differs from *M. baolongensis*, *M. nankiangensis*, *M. pachyproctus*, *M. shuichengensis* and *M. tuberogranulata* Shen, Mo & Li, 2010 (vs. just reaching posterior corner of the eye in the latter); differs from *M. daweimontis*, *M. glandulosa*, *M. lini*, *M. major*, *M. medongensis*, *M. obesa*, and *M. sangzhiensis* (vs. reaching the anterior corner of the eye or beyond eye or nostril and tip of snout in the latter); differs from *M. leishanensis* Li, Xu, Liu, Jiang, Wei & Wang, 2018 (vs. reaching middle part of eye in this group of species); and differs from *M. mufumontana* (vs. reaching tympanum in males and to the eye in females).

By having an internal single subgular vocal sac in male, *Megophrys chishuiensis* sp. nov. differs from *M. caudoprocta*, *M. shapingensis*, and *M. shuichengensis* (vs. vocal sac absent).

By having nuptial pads and nuptial spines on dorsal surface of the base of the first two fingers in breeding males, *Megophrys chishuiensis* sp. nov. differs from *M. acuta*, *M. feii*, *M. shapingensis*, and *M. shuichengensis* (vs. lacking in these species).

The congeners *M. carinense*, *M. jiangi*, *M. leishanensis*, *M. liboensis*, *M. shuichengensis*, and *M. spinata* have sympatric distribution with *Megophrys chishuiensis* sp. nov. (Fei et al. 2012; Zhang et al. 2017; Li et al. 2018; Liu et al. 2020). The new species can be distinguished from these species by a series of morphological characters as follows. The new species vs. *M. carinense*: body size smaller (adult males with 43.4–44.1 mm and adult females with SVL 44.8–49.8 mm vs. adult males with 92–123 mm and adult females with SVL 137mm), vomerine teeth absent (vs. present), horn-like tubercle at the edge of each upper eyelid small (vs. prominent), tongue not notched behind (vs. notched behind), lacking lateral fringe in toes (vs. present), and toes without webs at bases (vs. one-fourth webbed). The new species vs. *M. jiangi*: body size bigger (adult males with 43.4–44.1 mm and adult females with SVL 44.8–49.8 mm vs. adult males with 34.4–39.2 mm and adult females with SVL 39.5–40.4 mm), and relative finger lengths II < I < V < III vs. I < II < V < III. The new species vs. *M. leishanensis*: body size bigger (adult males with 43.4–44.1 mm and adult females with SVL 44.8–49.8 mm vs. adult males with 30.4–38.7 mm and adult females with SVL 42.3 mm), and tibiotarsal articulation reaching forward to the region between tympanum and eye when hindlimb is stretched along the side of the body vs. reaching middle part of eye. The new species vs. *M. liboensis*: body size smaller in adult females (adult females with SVL 44.8–49.8 mm vs. adult females with SVL 60.8–70.6 mm), vomerine teeth absent vs. vomerine teeth present, and horn-like tubercle at the edge of each upper eyelid is small vs. prominent. The new

species vs. *M. shuichengensis*: body size smaller (adult males with 43.4–44.1 mm and adult females with SVL 44.8–49.8 mm vs. adult males with 102.0–118.3 mm and adult females with SVL 99.8–115.6 mm), horn-like tubercle at the edge of each upper eyelid is small vs. prominent, tongue not notched behind vs. tongue notched behind, lacking lateral fringe in toes vs. present, toes without webs at bases vs. one-fourth webbed, having an internal single subgular vocal sac in male vs. absent, and having nuptial pads and nuptial spines on the dorsal base of the first two fingers in breeding male vs. lacking. The new species vs. *M. spinata*: body size is smaller (adult males with 43.4–44.1 mm and adult females with SVL 44.8–49.8 mm vs. adult males with 47.2–54.4 mm and adult females with SVL 54.0–55.0 mm), horn-like tubercle at the edge of each upper eyelid is small vs. lacking tubercle, tongue not notched behind vs. notched behind, lacking lateral fringe in toes vs. present, and toes without webs at bases vs. one-fourth webbed.

Megophrys chishuiensis sp. nov. is phylogenetically closest to *M. minor*, and this new species could be identified from the latter distinctly by having larger body size (SVL 43.4–44.1 mm in males vs. 34.5–41.2 mm in males of *M. minor*), having a small horn-like tubercle at the edge of each upper eyelid (vs. absent in the latter), tongue not notched behind (vs. notched in the latter), tibiotarsal articulation reaching the level between tympanum to eye when leg stretched forward (vs. reaching the level between eye and tip of snout in the latter), and having two metatarsal tubercles in each hand (vs. absent in the latter).

Distribution and habitats. *Megophrys chishuiensis* sp. nov. is known from the type locality, Chishui National Nature Reserve (28.38–28.45N, 106.05–109.75E), Chishui City, Guizhou Province, China at elevations between 270–604 m. The individuals of the new species were frequently found in bamboo forest nearby the streams (Fig. 8), and five sympatric amphibian species were also found: *Megophrys omeimontis*, *Odorrana margaratae* (Liu, 1950), *Zhangixalus omeimontis* (Stejneger, 1924), and *Rana omeimontis* Ye & Fei, 1993.

Etymology. The specific name *chishuiensis* refers to the distribution of this species, Chishui City, Guizhou Province, China. We propose the common name "Chishui horned toad" and its Chinese name as Chi Shui Jiao Chan (赤水角蟾).

Figure 8. Habitats of *Megophrys chishuiensis* sp. nov. in the type locality, Chishui National Nature Reserve, Chishui City, Guizhou Province, China **A** landscape of montane forests **B** a mountain stream (the inset illustrates the holotype on stone).

Discussion

The new species, *Megophrys chishuiensis* sp. nov., resembles *M. minor* and *M. jiangi*, and detailed comparison with different data sets are important for recognizing them. Our molecular phylogenetic data on mitochondrial DNA and nuclear DNA, and morphological comparisons both separated the new species from the two closely related species. *Megophrys minor* were reported to be distributed widely through the provinces of Sichuan, Guizhou, Chongqing, Yunnan, Guangxi, Jiangxi and north of Vietnam (Fei et al. 2012), but detailed investigations with multiple data suggested that several populations of the species should contain cryptic species (including *Megophrys chishuiensis* sp. nov. and *M. jiangi*). In recent years, a lot of new species of the genus *Megophrys* have been gradually described, of which, a large part of number of species were found in China (Frost 2020). To now, among the 97 species of *Megophrys*, 51 species were discovered in China. Even so, dozens of cryptic species need to be described (Chen et al. 2016; Liu et al. 2018) just in China. Obviously, we should conduct more investigations on the differentiation of the populations and explore the species identity in the wide range.

Megophrys chishuiensis sp. nov. with a narrow distribution also fits the "micro-endemism" model like many other congeners (Liu et al. 2018; Wang et al. 2019). Besides, the new species is likely to be threatened by several factors, i.e., developing tourism in Chishui National Nature Reserve, constructions in this area and increasing pollution from tourists. Reasonable managements of tourism in this area may probably facilitate the protection of the populations of the toad and other animal species.

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References

Beddard FE (1908) Contributions to the knowledge of the anatomy of the batrachian family Pelobatidae. Proceedings of the Zoological Society of London 1907: 871–891. https://doi.org/10.1111/j.1469-7998.1907.tb06963.x

Boulenger GA (1886) Description of a new frog of the genus *Megalophrys*. Proceedings of the Zoological Society of London 1885: 1–850.

Boulenger GA (1887) Description of a new frog of the genus *Megalophrys*. Annali del Museo Civico di Storia Naturale di Genova Serie 2, 4: 512–513.

Boulenger GA (1889) Description of a new batrachian of the genus *Leptobrachium*, obtain by M. L. Burma. Annali del Museo Civico di Storia Naturale di Genova. Serie 2, 7: 748–750.

Boulenger GA (1893) Descriptions of three new batrachians from Tonkin. Annals and Magazine of Natural History, Series 7(12): 186–188. https://doi.org/10.1080/00222930308678835

Boulenger GA (1899a) Descriptions of three new reptiles and a new batrachian from Mount Kina Balu, North Borneo. Annals and Magazine of Natural History, Series 7(4):1–453. https://doi.org/10.1080/00222939908678228

Boulenger GA (1899b) On a collection of reptiles and batrachians made by Mr. J. D. La Touche in N.W. Fokien, China. Proceedings of the Zoological Society of London 1899: 159–172.

Boulenger GA (1903) Report on the batrachians and reptiles. Annandale, N., and H. C. Robinson eds., Fasciculi Malayenses. Anthropological and Zoological Results of an Expedition to Perak and the Siamese Malay States 1901–1903 undertaken by Nelson Annandale and Herbert C. Robinson under the auspices of the University of Edinburgh and the University of Liverpool. Volume 2, Zoology, Part 1: 131–176.

Boulenger GA (1908) A revision of the oriental pelobatid batrachians (genus *Megophrys*). Proceedings of the Zoological Society of London 78(2): 407–430. https://doi.org/10.1111/j.1096-3642.1908.tb01852.x

Bourret R (1937) Notes herpétologiques sur l'Indochine française. XIV. Les batraciens de la collection du Laboratoire des Sciences Naturelles de l'Université. Descriptions de quinze espèces ou variétés nouvelles. Annexe au Bulletin Général de l'Instruction Publique Hanoi, 1937: 5–56.

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Phylogenetics and Evolution 17: 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Chen JM, Zhou WW, Poyarkov Jr NA, Stuart BL, Brown RM, Lathrop A, Wang YY, Yuan ZL, Jiang K, Hou M, Chen HM, Suwannapoom C, Nguyen SN, Duong TV, Papenfuss TJ, Murphy RW, Zhang YP, Che J (2017) A novel multilocus phylogenetic estimation reveals unrecognized diversity in Asia toads, genus *Megophrys* sensu lato (Anura: Megophryidae). Molecular Phylogenetics and Evolution 106: 28–43. https://doi.org/10.1016/j.ympev.2016.09.004

Deuti K, Grosjean S, Nicolas V, Vasudevan K, Ohler A (2017) Nomenclatural puzzle in early *Megophrys* nomina (Anura, Megophryidae) solved with description of two new species from India (Darjeeling hills and Sikkim). Alytes 34: 20–48.

Dubois A (1987) Miscellanea taxinomica batrachologica (I). Alytes 1987[1986]: 7–95.

Dubois A, Ohler A (1998) A new species of *Leptobrachium* (*Vibrissaphora*) from northern Vietnam, with a review of the taxonomy of the genus *Leptobrachium* (Pelobatidae, Megophryinae). Dumerilia 4(14): 1–32.

Fei L, Hu SQ, Ye CY, Huang YZ (2009) Fauna Sinica. Amphibia (Vol. 2). Anura. Science Press, Beijing, 328–481. [In Chinese]

Fei L, Ye CY, Huang YZ (1983) Two new subspecies of *Megophrys omeimontis* Liu from China (Amphibia, Pelobatidae). Acta Herpetologica Sinica. New Series, Chengdu 2(2): 49–52. [In Chinese with English abstract]

Fei L, Ye CY, Huang YZ (1990) Key to Chinese Amphibians. Publishing House for Scientific and Technological, Chongqing, China, 96–103. [In Chinese]

Fei L, Ye CY, Huang YZ (2001) The Colour Handbook of the Amphibians of Sichuan, Science Press, Beijing, China, 141–151. [In Chinese]

Fei L, Ye CY (2005) Two new species of Megophryidae from China. In: Fei et al. (Ed.) The Key and Illustration of Chinese. Sichuan Publishing House of Science and Technology, Chongqing, China, 253–255. [In Chinese]

Fei L, Ye CY, Jiang JP (2012) Colored atlas of Chinese Amphibians and their distributions. Sichuan Publishing House of Science and Technology, Chengdu, 135–247. [In Chinese]

Fei L, Ye CY, Jiang JP (2016) Genus *Liuophrys* Fei, Ye and Jiang, new genus; Subgenus *Atympanophrys* (*Borealophrys*) Fei, Ye and Jiang, new subgenus; Subgenus *Atympanophrys* (*Gigantophrys*) Fei, Ye and Jiang, new subgenus; Genus *Boulenophrys* Fei, Ye and Jiang, 2016, new genus; Subgenus *Xenophrys* (*Tianophrys*) Fei, Ye and Jiang, new subgenus. In: Fei L, Ye CY (Eds) Amphibians of China (Vol. I). Science Press, Beijing, 611–735.

Frost DR (2020) Amphibian Species of the World: an Online Reference. Version 6.0. Electronic Database. American Museum of Natural History, New York. http://research.amnh.org/herpetology/amphibia/index.html [Accessed on: 2020-4-20]

Günther ACLG (1864) The reptiles of British India. Ray Society. London, 414 pp.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59(3): 07–321. https://doi.org/10.1093/sysbio/syq010

Hall TA (1999) BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41(41): 95–98.

Hu SX, Zhao EM, Liu CZ (1966) A herpetological survey of the Tsinling and Ta-pa shan region. Acta Zoologica Sinica 18(1): 57–89.

Hu SX, Zhao EM, Liu CZ (1973) A survey of amphibians and reptiles in Kweichow province, including a herpetofauna analysis. Acta Zoologica Sinica 19(2): 149–171.

Huang YZ, Fei L (1981) Two new species of amphibians from Xizang. Acta Zootaxonomica Sinica 6: 211–215.

Inger RF (1989) Four new species of frogs from Borneo. Malayan Nature Journal. Kuala Lumpur 42: 229–243.

Inger RF, Romer JD (1961) A new pelobatid frog of the genus *Megophrys* from Hong Kong. Fieldiana. Zoology 39(46): 533–538. https://doi.org/10.5962/bhl.title.3373

Inger RF, Stuebing RB, Lian TF (1995) New species and new records of Anurans from Boreno. Raffles Bulletin of Zoology, Singapore 43(1): 115–131.

Inger RF, Iskandar DT (2005) A collection of amphibians from west Sumatra, with description of a new species of *Megophrys* (Amphibia: Anura). Raffles Bulletin of Zoology, Singapore 133–142.

Jiang JP, Ye CY, Fei L (2008) A New Horn Toad *Megophrys sangzhiensis* from Hunan, China (Amphibia, Anura). Zoological Research 29(2): 219–222. [In Chinese with English abstract] https://doi.org/10.3724/SP.J.1141.2008.00219

Kuhl H, Van Hasselt JC (1822) Uittreksels uit breieven van de Heeren Kuhl en van Hasselt, aan de Heeren C. J. Temminck, Th. van Swinderen en W. de Haan. Algemeene Konst-en Letter-Bode 7: 99–104.

Lathrop A (1997) Taxonomic review of the megophryid frogs (Anura: Pelobatoidea). Asian Herpetological Research 7: 68–79. https://doi.org/10.5962/bhl.part.18857

Lemmon AR, Moriarty EC (2004) The importance of proper model assumption in Bayesian phylogenetics. Systematic Biology 53(2): 265–277. https://doi.org/10.1080/10635150490423520

Li YL, Jin MJ, Zhao J, Liu ZY, Wang YY, Pang H (2014) Description of two new species of the genus *Megophrys* (Amphibia: Anura: Megophryidae) from Heishiding Nature Reserve, Fengkai, Guangdong, China, based on molecular and morphological data. Zootaxa 3795(4): 449–471. https://doi.org/10.11646/zootaxa.3795.4.5

Li SZ, Xu N, Liu J, Jiang JP, Wei G, Wang B (2018) A new species of the Asian toad genus *Megophrys sensu lato* (Amphibia: Anura: Megophryidae) from Guizhou Province, China. Asian Herpetological Research 9(4): 224–239. https://doi.org/10.16373/j.cnki.ahr.180072

Li Y, Zhang DD, Lyu ZT, Wang J, Li YL, Liu ZY, Chen HH, Rao DQ, Jin ZF, Zhang CY, Wang YY (2020) Review of the genus *Brachytarsophrys* (Anura: Megophryidae), with revalidation of *Brachytarsophrys platyparietus* and description of a new species from China. Zoological Research 41(2): 105–122. https://doi.org/10.24272/j.issn.2095-8137.2020.033

Liu CZ (1950) Amphibians of Western China. Fieldiana. Zoology Memoires 2: 1–400. https://doi.org/10.5962/bhl.part.4737

Liu CZ, Hu SQ, Yang HH (1960) Amphibian of Yunnan collected in 1958. Acta Zoological Sinica 12(2): 149–174.

Liu ZY, Zhu TQ, Zeng ZC, Lyu ZT, Wang J, Messenger K, Greenberg AJ, Gou ZX, Yang ZH, Shi SH, Wang YY (2018) Prevalence of cryptic species in morphologically uniform taxa-Fast speciation and evolutionary radiation in Asian frogs. Molecular Phylogenetics and Evolution 127: 723–731. https://doi.org/10.1016/j.ympev.2018.06.020

Liu J, Li SZ, Wei G, Xu N, Cheng YL, Wang B, Wu J (2019) A new species of the Asian toad genus *Megophrys sensu lato* (Anura: Megophryidae) from Guizhou Province, China. Asian Herpetological Research 11(1): 1–18. https://doi.org/10.16373/j.cnki.ahr.190041

Mahony S (2011) Two new species of *Megophrys* Kuhl & van Hasselt (Amphibia: Megophryidae), from western Thailand and southern Cambodia. Zootaxa 2734: 23–39. https://doi.org/10.11646/zootaxa.2734.1.2

Mahony S, Sengupta S, Kamei RG, Biju SD (2011) A new low altitude species of *Megophrys* Kuhl and van Hasselt (Amphibia: Megophryidae), from Assam, Northeast India. Zootaxa 3059: 36–46. https://doi.org/10.11646/zootaxa.3059.1.2

Mahony S, Teeling EC, Biju SD (2013) Three new species of horned frogs, *Megophrys* (Amphibia: Megophryidae), from northeast India, with a resolution to the identity of *Megophrys boettgeri* populations reported from the region. Zootaxa 3722(2): 143–169. https://doi.org/10.11646/zootaxa.3722.2.2

Mahony S, Nicole MF, Biju SD, Teeling EC (2017) Evolutionary history of the Asian Horned Frogs (Megophryinae): integrative approaches to timetree dating in the absence of a fossil record. Molecular Phylogenetics and Evolution 34(3): 744–771. https://doi.org/10.1093/molbev/msw267

Mahony S, Kamei RG, Teeling EC, Biju SD (2018) Cryptic diversity within the *Megophrys major* species group (Amphibia: Megophryidae) of the Asian Horned Frogs: Phylogenetic perspectives and a taxonomic revision of South Asian taxa, with descriptions of four new species. Zootaxa 4523: 1–96. https://doi.org/10.11646/zootaxa.4523.1.1

Malkmus R, Matsui M (1997) *Megophrys kobayashii*, ein neuer pelobatider Frosch vom Mount Kinabalu. Sauria, Berlin 19: 31–37.

Mathew R, Sen N (2007) Description of two new species of *Megophrys* (Amphibia: Anura: Megophryidae) from North-east India. Cobra 1: 18–28.

McGuire JA, Witt CC, Altshuler DL, Remsen JV (2007) Phylogenetic systematics and biogeography of hummingbirds: Bayesian and maximum likelihood analyses of partitioned data and selection of an appropriate partitioning strategy. Systematic Biology 56(5): 837–856. https://doi.org/10.1080/10635150701656360

Messenger KR, Dahn HA, Liang YR, Xie P, Wang Y, Lu CH (2019) A new species of the genus *Megophrys* Gunther, 1864 (Amphibia: Anura: Megophryidae) from Mount Wuyi, China. Zootaxa 4554(2): 561–583. https://doi.org/10.11646/zootaxa.4554.2.9

Mo XY, Shen YH, Li HH, Wu MS (2010) A new species of *Megophrys* (Amphibia: Anura: Megophryidae) from the northwestern Hunan Province, China. Current Zoology 56(4): 432–436. https://doi.org/10.1093/czoolo/56.4.432

Munir M, Hamidy A, Farajallah A, Smith EN (2018) A new *Megophrys* Kuhl and Van Hasselt (Amphibia: Megophryidae) from southwestern Sumatra, Indonesia. Zootaxa 4442: 389–412. https://doi.org/10.11646/zootaxa.4442.3.3

Munir M, Hamidy A, Matsui M, Iskandar DT, Sidik I, Shimada T (2019) A new species of *Megophrys* Kuhl & Van Hasselt (Amphibia: Megophryidae) from Borneo allied to *M. nasuta* (Schlegel, 1858). Zootaxa 4679: 1–24. https://doi.org/10.11646/zootaxa.4679.1.1

Nguyen TQ, Pham CT, Nguyen TT, Luong AM, Ziegler T (2020) A new species of *Megophrys* (Amphibia: Anura: Megophryidae) from Vietnam. Zootaxa 4722: 401–422. https://doi.org/10.11646/zootaxa.4722.5.1

Ohler A, Swan SR, Daltry JC (2002) A recent survey of the amphibian fauna of the Cardamom Mountains, Southwest Cambodia with descriptions of three new species. Raffles Bulletin of Zoology, Singapore 50(2): 465–481.

Ohler A (2003) Revision of the genus *Ophryophryne* Boulenger, 1903 (Megophryidae) with description of two new species. Alytes 21(1): 23–44.

Orlov NL, Pyarkov Jr NA, Nguyen TT (2015) Taxonomic notes on *Megophrys* frogs (Megophryidae: Anura) of Vietnam, with description of a new species. Russian Journal of Herpetology 22: 206–218.

Pope CH (1929) Four new frogs from Fukien Province, China. American Museum Novitates 352: 1–5.

Poyarkov NA, Duong Jr TV, Orlov NL, Gogoleva SI, Vassilieva AB, Nguyen LT, Nguyen VDH, Nguyen SN, Che J, Mahony S (2017) Molecular, morphological and acoustic assessment of the genus *Ophryophryne* (Anura, Megophryidae) from Langbian Plateau, southern Vietnam, with description of a new species. ZooKeys 672: 49–120. https://doi.org/10.3897/zookeys.672.10624

Rao DQ, Yang DT (1997) The karyotypes of Megophryinae (Pelobatidae) with a discussion on their classification and phylogenetic relationships. Asian Herpetological Research 7: 93–102. https://doi.org/10.5962/bhl.part.18858

Robert L, Brett C, Simon YWH, Stephane G (2012) PartitionFinder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses. Molecular Phylogenetics and Evolution 29(6): 1695–1701. https://doi.org/10.1093/molbev/mss020

Ronquist FR, Huelsenbeck JP (2003) MrBayes3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19(12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York, 1659 pp.

Schlegel H (1858) Handleiding tot de Beoefening der Dierkunde (Vol. 2). Koninklijke Militaire Akademie, Breda, 57 pp. https://doi.org/10.5962/bhl.title.11648

Shen MM, Liang D, Feng YJ, Chen MY, Zhang P (2013) A versatile and highly efficient toolkit including 102 nuclear markers for Vertebrate phylogenomics, tested by resolving the higher level relationships of the Caudata. Molecular Phylogenetics and Evolution 30(10): 2235–2248. https://doi.org/10.1093/molbev/mst122

Shen YH (1994) A new pelobatid toad of the genus *Megophrys* from China (Anura: Pelobatidae). In: Zoological Society of China (China Zoological Society, Institute of zoology, Chinese academy of sciences, School of life sciences, Peking University, Department of biological science and technology, Tsinghua university) The 60th Anniversary of the Foundation of the Zoological Society of China, Nanking (China), September 1994. China Science and Technology Publishing House, 603–606.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87(6): 651–701. https://doi.org/10.1093/aesa/87.6.651

Sjöander K, Beskow J (2000) Wavesurfer (Anura: Pelobatidae). Acoustical society of China Editor. The International Conference on Spoken Language Processing, Beijing (China), October 2000. Military Yiwen Publishing House, 464–467.

Smith MA (1921) New or little-known reptiles and batrachians from southern Annam (Indo-China). Proceedings of the Zoological Society of London 1921: 423–440. https://doi.org/10.1111/j.1096-3642.1921.tb03271.x

Stejneger L (1926) Two new tailless amphibians from western China. Proceedings of the Biological Society of Washington 39: 53–54.

Stuart BL, Chuaynkern Y, Chan-ard T, Inger RF (2006) Three new species of frogs and a new tadpole from eastern Thailand. Fieldiana Zoology, New Series 1543: 1–10. https://doi.org/10.3158/0015-0754(2006)187[1:TNSOFA]2.0.CO;2

Tamura K, Stecher G, Peterson D, Fiipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis, version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Tapley B, Cutajar T, Mahony S, Nguyen CT, Dau VQ, Nguyen TT, Luong HV, Rowley JJL (2017) The Vietnamese population of *Megophrys kuatunensis* (Amphibia: Megophryidae) represents a new species of Asian horned frog from Vietnam and southern China. Zootaxa 4344(3): 465–492. https://doi.org/10.11646/zootaxa.4344.3.3

Tapley B, Cutajar TP, Mahony S, Nguyen CT, Dau VQ, Luong AM, Le DT, Nguyen TT, Nguyen TQ, Portway C, Luong HV, Rowley JJL (2018) Two new and potentially highly threatened *Megophrys* Horned frogs (Amphibia: Megophryidae) from Indochina's highest mountains. Zootaxa 4508: 301–333. https://doi.org/10.11646/zootaxa.4508.3.1

Taylor EH (1920) Philippine Amphibia. Philippine Journal of Science 16: 213–359. https://doi.org/10.5962/bhl.part.4751

Tian YZ, Sun A (1995) A new species of *Megophrys* from China (Amphibia: Pelobatidae). Journal of Liupanshui Teachers College 52(3): 11–15. [In Chinese]

Tian WS, Hu QX (1983) Taxonomic study on genus *Megophrys*, with descriptions of two genera. Acta Herpetologica Sinica 2: 41–48.

Vieites DR, Min MS, Wake DB (2007) Rapid diversification and dispersal during periods of global warming by plethodontid salamanders. Proceedings of the National Academy of Sciences of the United States of America 104: 19903–19907. https://doi.org/10.1073/pnas.0705056104

Wang J, Liu ZY, Lyu ZT, Wang YY (2017a) A new species of the genus *Megophrys* (Amphibia: Anura: Megophryidae) from an offshore island in Guangdong Province, southeastern China. Zootaxa 4324(3): 541–556. https://doi.org/10.11646/zootaxa.4324.3.8

Wang YE, Liu BQ, Jiang K, Jin W, Xu JN, Wu CH (2017b) A new species of the Horn Toad of the genus *Xenophrys* from Zhejiang, China (Amphibia: Megophryidae). Chinese Journal of Zoology 52: 19–29. [in Chinese with English abstract]

Wang YY, Zhang TD, Zhao J, Sung YH, Yang JH, Pang H, Zhang Z (2012) Description of a new species of the genus *Megophrys* Günther, 1864 (Amphibia: Anura: Megophryidae) from Mount Jinggang, China, based on molecular and morphological data. Zootaxa 3546: 53–67. https://doi.org/10.11646/zootaxa.3546.1.4

Wang YY, Zhao J, Yang JH, Zhou ZM, Chen GL, Liu Y (2014) Morphology, molecular genetics, and bioacoustics support two new sympatric *Megophrys* (Amphibia: Anura Megophryidae) species in Southeast China. Plos ONE 9: e93075. https://doi.org/10.1371/journal.pone.0093075

Wang L, Deng XJ, Liu Y, Wu QQ, Liu Z (2019a) A new species of the genus *Megophrys* (Amphibia: Anura: Megophryidae) from Hunan, China. Zootaxa 4695(4): 301–330. https://doi.org/10.11646/zootaxa.4695.4.1

Wang J, Lyu ZT, Liu ZY, Liao CK, Zeng ZC, Li YL, Wang YY (2019b) Description of six new species of the subgenus *Panophrys* within the genus *Megophrys* (Anura, Megophryidae) from southeastern China based on molecular and morphological data. ZooKeys 851: 113–164. https://doi.org/10.3897/zookeys.851.29107

Wang B, Wu YQ, Peng JW, Shi SC, Lu NN, Wu J (2020) A new *Megophrys* Kuhl and Van Hasselt (Amphibia: Megophryidae) from southeastern China. ZooKeys 851: 113–164. https://doi.org/10.3897/zookeys.904.47354

Wu YH, Suwannapoom C, Poyarkov Jr NA, Chen JM, Pawangkhanant P, Xu K, Jin JQ, Murphy RW, Che J (2019) A new species of the genus *Xenophrys* (Anura: Megophryidae) from northern Thailand. Zoological Research 40: 564–574. https://doi.org/10.24272/j.issn.2095-8137.2019.032

Yang JH, Wang J, Wang YY (2018) A new species of the genus *Megophrys* (Anura: Megophryidae) from Yunnan Province, China. Zootaxa 4413: 325–338. https://doi.org/10.11646/zootaxa.4413.2.5

Ye CY, Fei L (1992) A new Pelobatid toda of the genus *Megophrys* from Xizang, China. Acta Herpetologica Sinica 1–2: 50–52. [In Chinese]

Ye CY, Fei L (1995) Taxonomic studies on the small type *Megophrys* in China including descriptions of the new species (subspecies) (Pelobatidae: genus *Megophrys*). Herpetologica Sinica 4–5: 72–81. [In Chinese]

Ye CY, Fei L, Xie F (2007) A new species of Megophryidae *Megophrys baolongensis* from China (Amphibia, Anura). Herpetologica Sinica 11: 38–41. [In Chinese]

Zhang Y, Li G, Xiao N, Li J, Pan T, Wang H, Zhang B, Zhou J (2017) A new species of the genus *Megophrys* (Amphibia: Anura: Megophryicae) from Libo County, Guizhou, China. Asian Herpetological Research 8: 75–85. https://doi.org/10.16373/j.cnki.ahr.160041

Zhao J, Yang JH, Chen GL, Chen CQ, Wang YY (2014) Description of a new species of the genus *Brachytarsophrys* Tian and Hu, 1983 (Amphibia: Anura: Megophryidae) from Southern China based on molecular and morphological data. Asian Herpetological Research 5(3): 150–160. https://doi.org/10.3724/SP.J.1245.2014.00150

Supplementary material 1

Table S1

Authors: Ning Xu, Shi-Ze Li, Jing Liu, Gang Wei, Bin Wang

Explanation note: Measurements of the adult specimens of *Megophrys chishuiensis* sp. nov.

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Link: https://doi.org/10.3897/zookeys.943.50343.suppl1

Supplementary material 2

Table S2

Authors: Ning Xu, Shi-Ze Li, Jing Liu, Gang Wei, Bin Wang

Explanation note: Localities, voucher information, and GenBank accession numbers for molecular samples used in this study.

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Supplementary material 3

Table S3

Authors: Ning Xu, Shi-Ze Li, Jing Liu, Gang Wei, Bin Wang

Explanation note: Primer sequences used in this study.

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Supplementary material 4

Table S4

Authors: Ning Xu, Shi-Ze Li, Jing Liu, Gang Wei, Bin Wang

Explanation note: Uncorrected p-distances between the *Megophrys* species based on the 16S gene sequences.

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